site is reprotonated from Asp96 (1). A transient water chain between asp 96 and the central binding site evolves on the milli-second time-scaleand the proton is transferred from asp 96 to the central binding site and the pump is reset (4). In summary, the emerging paradigm invokes the principle that protein bound water molecules are as functional as amino acids residues (2).

*References*

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**1862-Symp**
**Progress Towards the Molecular Mechanism of Complex I**
**Judy Hirst**.
Medical Research Council, Cambridge, United Kingdom.

Complex I (NADH:ubiquinone oxidoreductase) is crucial to respiration in many aerobic organisms. In mitochondria it oxidises NADH (regenerating NAD+ for the tricarboxylic acid cycle and fatty-acid oxidation), reduces ubiquinone (the electrons are then used to reduce oxygen to water), and transports protons across the mitochondrial inner membrane (contributing to the proton motive force that supports ATP synthesis and transport processes). Complex I is also a major contributor to cellular reactive oxygen species production.

The mechanism of complex I comprises four 'sequential' steps. NADH oxidation by the flavin mononucleotide, and intramolecular electron transfer from the flavin to bound quinone (along a chain of iron-sulphur clusters), are increasingly well understood. Conversely, the mechanisms of quinone reduction and proton translocation remain poorly defined, although recent structural analyses of the membrane domain of complex I (R. G. Efremov, R. Baradaran & L. A. Sazanov (2010) Nature 465, 441-7) have revealed intriguing features, including a lateral helix running in the plane of the membrane, and an elevated position for the proposed quinone binding site, above the membrane plane. This talk will present and discuss current strategies and recent data to address the mechanisms of quinone reduction and proton translocation by complex I.

**1863-Symp**
**Structural Basis and Mechanism of Proton Translocation in Complex I and Complex III**
**Carola Hunte**.
University of Freiburg, Freiburg, Germany.

Proton pumping respiratory complexes drive the energy conversion in cellular respiration by coupling electron and proton transfer via defined mechanisms. In complex I and complex III, bypass reactions result in production of reactive oxygen species (ROS) which are implicated in diseases and physiological processes of aging. Structure and Q cycle mechanism of complex III are in general well described [1]. Yet, several key features of the mechanism, such as the oxidation of ubiquinol, pathways for proton uptake and release and ROS production are still in question and will be discussed. Elucidation of structure and mechanism of complex I is only at its beginning. X-ray crystallographic analysis of the largest and most complicated respiratory membrane protein complex revealed the structural basis for a new mechanism of proton pumping by conformational coupling [2]. Data of the structural analysis will be presented.

*References*

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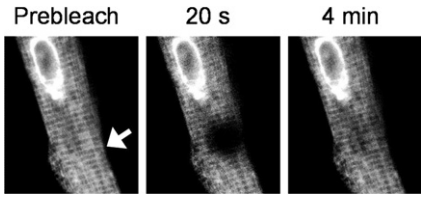
**1864-Symp**
**Proton Pumping and Energy Transduction in Cytochrome C Oxidase**
**Gerhard Hummer**.
National Institute of Health, Bethesda, MD, USA.

In my talk I will describe how simple physical processes contribute to the function of cytochrome c oxidase, the enzyme that powers aerobic life. Oxidase is responsible for the conversion of chemical energy from food into the electrochemical gradient that drives the synthesis of ATP. As the biological "fuel cell," it reduces oxygen to water and uses the released energy to pump protons across a membrane. With the help of theory and simulation, we could show how the chemical energy of oxygen reduction is harnessed to move protons against an electrochemical gradient without violation of the second law of thermodynamics, and how the unique dynamic and thermodynamic properties of water at the nanoscale are exploited.

## Platform AG: Cardiac Muscle I

**1865-Plat**
**Fret Measurements in Electrically Paced Adult Cardiac Myocytes Suggest the Phospholamban-SERCA2a Regulatory Complex is not Dissociated by Beat-To-Beat Elevations of Cytosolic Calcium**
**Daniel J. Blackwell**, Philip Bidwell, Seth L. Robia.
Loyola University Chicago, Maywood, IL, USA.

To investigate the regulatory interaction between cardiac sarco/endoplasmic reticulum calcium ATPase (SERCA2a) and phospholamban (PLB), we expressed Cerulean-SERCA2a and YFP-PLB in adult rabbit cardiac muscle cells using adenovirus vectors. Confocal microscopy showed a fluorescence pattern of striations and longitudinal streaks indicating SERCA and PLB were correctly localized in the sarcoplasmic reticulum (SR). Bright perinuclear fluorescence was also observed. Fluorescence recovery after photobleaching (FRAP) experiments showed that SERCA and PLB were mobile over multiple sarcomeres on a timescale of tens of seconds. The concentration dependence of SERCA-PLB fluorescence resonance energy transfer (FRET) showed maximum FRET (FRETmax) was 30%, and also yielded the apparent dissociation constant (Kd). Addition of thapsigargin increased the apparent Kd, suggesting a reduced affinity of PLB for the pump in the presence of the inhibitor. Pacing of myocytes did not result in large changes in SERCA-PLB FRET, suggesting that the regulatory complex is not disrupted by beat-to-beat elevations of cytosolic calcium. The data are compatible with parallel experiments in heterologous cells indicating that the PLB-SERCA interaction is reduced, but not abolished by thapsigargin and calcium.




**1866-Plat**
**Phosphorylation and Mutation Induce an Order-To-Disorder Transition in the Cytoplasmic Domain of Phospholamban**
John Paul Glaves[1], Catharine A. Trieber[1], Delaine K. Ceholski[2], David L. Stokes[3], **Howard S. Young**[1].
[1]National Institute for Nanotechnology & University of Alberta, Edmonton, AB, Canada, [2]University of Alberta, Edmonton, AB, Canada, [3]New York University & New York Structural Biology Center, New York, NY, USA.

Phospholamban physically interacts with the sarcoplasmic reticulum calcium pump (SERCA) and regulates contractility of the heart in response to adrenergic stimuli. We have studied this interaction using electron microscopy of two-dimensional crystals of SERCA in complex with phospholamban. In previous studies, phospholamban oligomers were found interspersed between SERCA dimer ribbons and a three-dimensional model was constructed to show interactions with SERCA. In the present study, we have examined the effects of phosphorylation and mutation of phospholamban on the interaction with SERCA in the two-dimensional crystals. Based on projection maps from negatively-stained and frozen-hydrated crystals, phosphorylation of Ser16 selectively disordered the cytoplasmic domain of wild-type phospholamban. This was not the case for a pentameric gain-of-function mutant (Lys27-to-Ala), which retained inhibitory activity and remained ordered in the phosphorylated state. A partial loss-of-function mutation that altered the charge state of phospholamban (Arg14-to-Ala) retained an ordered state, while a complete loss-of-function mutation (Asn34-to-Ala) was disordered. The functional state of phospholamban correlated with an order-to-disorder transition of phospholamban's cytoplasmic domain in the two-dimensional co-crystals. Furthermore, the residues studied

(Ser16, Lys27 and Asn34) may implicate domain Ib of phospholamban in the order-to-disorder transition. In summary, the two-dimensional co-crystals with SERCA require a functional pentameric form of phospholamban, which physically interacts with SERCA at an accessory site distinct from that used by the phospholamban monomer for the inhibitory association. Phosphorylation or mutation of phospholamban alters the SERCA-pentamer interaction in a manner normally associated with inhibition by the monomer.

**1867-Plat**

**Thrombospondin-4 is Necessary for the Increased Calcium Cycling Associated with the Slow Force Response**

**Jonathan A. Kirk**, David A. Kass, Oscar H. Cingolani.
Johns Hopkins University, Baltimore, MD, USA.

Thrombospondin-4 (TSP4) is a matricellular protein found in the heart and upregulated in heart failure; however, its role in cardiac regulation is unknown. Our previous work showed that mice lacking the TSP4 gene (*tsp4-/-)* respond normally to acute (seconds) pressure overload, but rapidly deteriorate minutes after. Thus, we hypothesize that TSP4 is involved in the slow force response (SFR), the second phase of stretch-mediated adaptation to loading, discovered a century ago, yet not fully understood. One proposed mechanism for the SFR is an increase in ERK phosphorylation that leads to increased $Na^+/H^+$ exchanger-1 activity. The latter increases intracellular $Na^+$, causing the $Na^+/Ca^{2+}$ exchanger (reverse mode) to rise intracellular $Ca^{2+}$, therefore increasing force. To investigate this, cardiac papillary muscles were isolated from *tsp4-/-* and wild-type (WT) littermates. Muscles were stretched from 92% of the length that generated maximum force ($L_{max}$ ) to 98% $L_{max}$. This length was maintained for 15 min while force and $Ca^{2+}$ transients (fura-2AM) were simultaneously recorded. The immediate rise in force without change in $Ca^{2+}$ (Frank-Starling) was similar in both groups. However, whereas a positive SFR occurred in WT (33 $\pm$ 7%, n=5), *tsp4-/-* muscle displayed a negative SFR ($-14$ $\pm$ 2%, n=5). The difference in force was accompanied by a rise in $Ca^{2+}$ in WT but not in *tsp4-/-* (7 $\pm$ 2% vs. 1$\pm$ 2%, $P<0.05$). Next, hearts were excised from mice 15 minutes following transaortic constriction (pressure overload) and phosphorylation of ERK1/2 and Akt was found to be decreased in the *tsp4-/-* mice ($P<0.05$). We conclude that TSP4 is necessary for cardiac adaptation to stretch. Its absence blunts the SFR (force and $Ca^{2+}$ rise). Moreover, TSP4 seems to be involved in this mechanism upstream of ERK1/2 and Akt.

**1868-Plat**

**Passive Properties of the Isolated Mouse Heart: Titin, Collagen and the Working Sarcomere Length Range**

**Charles S. Chung**, Nathan E. Cromer, Henk L. Granzier.
University of Arizona, Tucson, AZ, USA.

Understanding the passive properties of the left ventricle (LV) is important with the high and increasing prevalence of Diastolic Heart Failure. We examined the sources of passive pressure in the isolated mouse heart and its working sarcomere length (SL) range. A Langendorff perfused isolated heart system was used with a volume controlled balloon inserted in the LV of the mouse (C57BL/6). Hearts were paced and perfused with a Tyrode solution at constant perfusion pressure. The volume control system was used to measure Frank-Starling (FS) relationships. Hearts were then arrested and permeabilized by perfusing the heart with 1% triton in a relaxing solution followed by measurement of the FS relationship. The obtained passive pressure-volume curve for the permeabilizied heart was lower than that of the intact heart; analysis revealed that turgor from constant pressure perfusion of the intact heart is a major source of this difference. To determine the molecular basis of the passive pressure of the skinned heart, we used both myofilament extraction and trypsin degradation of titin and repeated FS measurements. Both methods suggest that titin produces ~80% of the passive pressure of the heart. Additional intact hearts were glutaraldehyde fixed at passive and BaCl2 activated conditions at similar volumes to the permeabilized hearts. Mid-wall fibers were carefully dissected from the hearts and SL measured via laser diffraction. SL of activated hearts ranged from 1.8-2.0um; diastolic SL from 1.95-2.15 um. Consistent with the isolated heart experiments, isolated muscle work shows that in this SL range, titin is the main contributor to passive tension. In conclusion, in the mouse LV diastolic sarcomere lengths exists in a relatively tight range and in this range titin is the major contributor to diastolic pressure of the intact heart.

**1869-Plat**

**Protein Phosphatase-5 is a Novel Titin Ligand Which Dephosphorylates Cardiac Titin and Reduces Passive Myocyte Stiffness**

Judith Krysiak, Nazha Hamdani, Sebastian Kötter, Patrick Lang, **Wolfgang A. Linke**.
Ruhr Univ. Bochum, Bochum, Germany.

Titin's elastic I-band region is a hotspot for protein-protein interactions and important for myocyte extensibility and passive stiffness. The titin springs can be phosphorylated at the N2B-unique sequence (N2Bus) by protein kinases (PK)A or PKG and at the PEVK-domain by PKCalpha, which affects passive stiffness. We searched for protein phosphatase(s) acting on the N2Bus. A yeast-2-hybrid (Y2H) screen with the human N2Bus ("bait") and a human heart cDNA library ("prey") detected the catalytic domain of the serine/threonine protein phosphatase-5 (PP5) as a binding partner of the cardiac N2Bus. The interaction was confirmed in forced Y2H screens with the N2Bus and full-length PP5 or PP5 catalytic subunit (PP5c), and also in GST-pulldown assays. In cardiomyocytes, PP5 was mainly in the cytosol but also in the nucleus and at the sarcomeric I-bands. Recombinant PP5 was found by autoradiography to dephosphorylate recombinant, PKG-phosphorylated, N2Bus, and PP5 bound more strongly to (PKA-/PKG-)phosphorylated N2Bus than to non-phosphorylated N2Bus. Phosphorylation of titin could be reduced in human heart tissue treated *ex vivo* with recombinant PP5c, an effect detected with phospho-N2Bus (S469) specific antibodies. A transgenic mouse model with PP5 overexpression revealed reduced cardiac titin phosphorylation levels compared to wildtype mouse hearts. PP5 expression was elevated in human heart failure, while titin phosphorylation was depressed. PP5c treatment of enzymatically skinned single human cardiomyocytes significantly reduced passive stiffness, which can potentially be explained by dephosphorylation of the PEVK-domain adjacent to the N2Bus. In conclusion, PP5 is a novel binding partner of cardiac titin at the N2Bus and acts to reduce passive myocyte stiffness by dephosphorylating the titin springs. PP5 may participate in mechanical signaling pathways converging on the titin springs.

**1870-Plat**

**Changes in Traction Force Mediated Through the Actin-Myosin Interface Controls Inside-Out Integrin Signaling Leading to Cardiac Hypertrophy**

**Milad Motarjemi**, Amanda Cline, Maria Valencik, Josh Baker.
University of Nevada School of Medicine, Reno, NV, USA.

In cardiac muscle, the interplay between forces generated by the contractile apparatus and sensed by integrins is thought to play an important role in cardiac remodeling and the pathogenesis of diseases such as hypertrophic cardiomyopathy (HCM). Although the effects of external forces on integrin signaling have been extensively studied in cardiac myocytes, the effects of internal contractile forces on integrin signaling remain undefined. To study the effects of altered contractility on integrin signaling, we have developed an *in vitro* tissue model consisting of a flexible micropattered scaffold which facilitates organized growth and differentiation of neonatal rat ventricular myocytes (NRVMs) into an adult phenotype with an organized filament structure (cardiofilaments) with intercalated discs. To measure integrin activation in this model system, we have constructed an adenovirus expressing a talin-GFP fusion protein. Binding of this talin-GFP to integrins upon integrin activation will be detected using total internal reflectance (TIRF) microscopy. We have confirmed talin-GFP colocalization with integrins at cell-ECM contacts using confocal microscopy. To measure traction forces generated by cardiofilaments, we use TIRF microscopy to measure displacements of fluorescent markers embedded within the laminin matrix. Simultaneous measurements of integrin activation and cardiofilament mechanics will allow us to directly determine how contractile forces altered by small molecule effectors such as blebbistatin influence integrin signaling. We hypothesize that activating sarcomere contractile forces will increase traction forces and activate integrins, initiating a hypertrophic response.

**1871-Plat**

**Reverse Ventricular Remodeling by Estrogen Therapy in a Rat Model of Experimental Pulmonary Hypertension**

**Rangarajan Nadadur**, Soban Umar, Rod Partow-Navid, Andrea Iorga, Humann Matori, Mansoureh Eghbali.
UCLA, Los Angeles, CA, USA.